Evaluation of the Microbial Growth Potential of Pharmaceutical Drug Products and Quality by Design

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ABSTRACT: The microbial growth potential of a pharmaceutical drug product refers to the ability of microorganisms to survive and proliferate in the product. Each drug formulation possesses a different potential for supporting or inhibiting microbial growth. Understanding this microbial growth potential can have a significant effect on the development and design of the drug manufacturing process. This article describes how this attribute can exert this effect on manufacturing process development and design through real examples and case studies obtained from the regulatory review of new drug and biologics license applications. In addition, this article describes how understanding the microbial growth potential of a pharmaceutical drug product is an element of the Quality by Design paradigm and how this understanding can simplify the drug development process and lead to better process design.

KEYWORDS: Quality by Design, QbD, Microbial growth, Pharmaceuticals, Water activity, New drug application, Biologics license application.

LAY ABSTRACT: The microbial growth potential of a pharmaceutical drug product refers to the ability of microorganisms to survive and proliferate in the product formulation. Each drug product formulation possesses a different potential for supporting or inhibiting microbial growth depending on its components. Understanding this microbial growth potential can have a significant effect on the development and design of the drug manufacturing process. This article describes how this attribute can affect manufacturing process development and design through real examples and case studies obtained from the regulatory review of new drug and biologics license applications. In addition, this article describes how understanding the microbial growth potential of a pharmaceutical drug product is an element of the Quality by Design paradigm and how this understanding can simplify the drug development process and lead to better process design.

Introduction

The microbial growth potential of a pharmaceutical drug product refers to the ability of microorganisms to survive and proliferate in the product. Each drug formulation possesses a different potential for supporting or inhibiting microbial growth. Growth of microorganisms can result in product degradation, product contaminated with toxins and impurities, loss of potency, unmet product specifications, and possibly regulatory action due to the potential impact on the patient. For these reasons, it is very important to evaluate the product microbial growth potential during drug development and prior to submitting an application to the Food and Drug Administration (FDA). The principles of Quality by Design (QbD) implore applicants to evaluate this attribute during product development because it can have a significant impact on the design of a terminal sterilization cycle or aseptic fill manufacturing process. Furthermore, it can be valuable information when designing an appropriate sampling plan to monitor microbiological attributes in the case of nonsterile dosage forms. The evaluation of product microbial growth potential is even more important for biological products such as monoclonal antibodies and other therapeutic proteins, which are by nature

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growth-promoting as they are produced from living organisms. Understanding the degree of microbial growth promotion for these biopharmaceuticals can have implications on the design of the manufacturing process and bioburden control prior to sterile filtration and aseptic fill.

While QbD is not a new concept in several industries (e.g., automobile and food industries), it has met with renewed interest and possible application in the pharmaceutical industry. FDA and industry have organized several bilateral meetings and workshops since 2005 (1) investing highly in its application to the manufacture of drug products. In addition, the FDA began the QbD pilot program for small molecule drugs in 2005 (2), while the corresponding program for therapeutic protein products began in 2008 (3). There have been multiple definitions for QbD (4). However, the authors define it as the development of a drug product formulation and the design of a manufacturing process using all current available knowledge to produce a pharmaceutical product with inherent qualities and properties for its intended use. Under the QbD paradigm, select studies can be performed to increase product knowledge for the production of a pharmaceutical with all the necessary attributes for its intended use. The principles of QbD are not limited to new products only, but can be applied to existing products and manufacturing processes. Improvements can be made continuously to a process and a product's performance. Continuous improvement results in consistent product quality, in reduction of the cost of quality, and is proactive rather than reactive in the prevention of mistakes and problems before they occur.

What is the relationship between microbial growth potential, product development, and QbD? What are the regulatory implications and benefits to pharmaceutical firms (if any)? This article attempts to answer these questions and provides several examples and case studies compiled from the review of new drug and biologics license applications submitted to the FDA. The examples and case studies further demonstrate the significance of the product microbial growth potential attribute and its promising impact on the quality of pharmaceutical drug products.

Microbial Growth Studies

Microbial growth studies can be performed in various ways, but the target is to obtain a basic understanding of the growth rate of select microorganisms in a drug product formulation. The product is inoculated with a low number of test microorganisms and aliquots are obtained at various intervals and storage conditions (e.g., room temperature, refrigeration conditions) to determine the microbial count. Further analysis and trending of the counts demonstrates whether the product formulation is bactericidal, bacteriostatic, or growth-promoting to each specific microorganism tested. The microorganisms tested should be selected after careful consideration of the manufacturing process, storage, and administration of the drug product. The authors further refer the reader to a recently published article (5) regarding the specifics of the microbial growth study that would satisfy FDA regulatory requirements. These studies are in essence growth curves of the selected test microorganisms in the product solution.

Water Activity as a Tool for Evaluating the Microbial Growth Potential of Nonsterile Pharmaceutical Dosage Forms

Microorganisms require water to grow. Water activity describes the amount of bound and unbound water available for chemical reactions. Thus, it can serve as a predictor of microbial growth potential; there is inhibition of microorganism growth when values are low while there is promotion when values are high. The food industry has used water activity as an indicator for predicting the microbial growth potential of food systems for decades. The application of this measurement tool in the pharmaceutical industry has only been implemented in the last few years. Cundell *et al.* (6) provide a survey of water activity values commonly found in pharmaceutical over-the-counter drug products. The importance of water activity is further demonstrated by the addition of Chapter ⟨1112⟩ Application of Water Activity Determination to Nonsterile Pharmaceutical Products in the *U.S. Pharmacopeia* (7, 8). Snider *et al.* (9) suggest the implementation of water activity testing in lieu of the Karl Fischer water testing method for solid oral dosage forms. Indeed, water activity can be a very useful indicator and a relatively easy test to perform for an initial assessment of a product's ability to support microbial growth. One must remember, however, that water activity does not imply the absence of microorganisms including spores. They may be present but not proliferating due to the adverse conditions. Thus, the measurement of water activity is not necessarily a reliable predictor of microbial degradation and stability of a product. If appropriate storage conditions are

not maintained (for example, temperature, humidity), microorganisms will survive at low water activity values only to proliferate when conditions become favorable. Besides these limitations, determining this attribute can lead to more effective sampling plans that consider only relevant types of microorganisms instead of those most commonly recommended by pharmacopeias. For example, it may be more relevant to test a tablet for molds rather than the presence of Gram-negative bacteria.

For nonsterile pharmaceutical products such as nonaqueous liquid formulations, ointments, and solid oral dosage forms (tablets, capsules, etc.), it is essential to evaluate water activity during the initial stages of product development. This information provides the rationale for decisions regarding the presence of a preservative system and its concentration, the design of the manufacturing process, the design of a scientific sampling plan for product release and stability testing of microbiological attributes, and determination of appropriate storage conditions and expiration dates. FDA International Conference on Harmonization (ICH) Guidance for Industry Q6A (10) includes a decision tree describing microbiological specifications for nonsterile dosage forms. The decision tree consists of helpful questions to aid in the performance of a suitable risk assessment for evaluating the probability of proliferation of microorganisms inherent in the product or derived from the manufacturing process.

Water activity data and results from microbial growth studies have led to reduced testing (or skip lot testing, where release testing is not required for each product lot) or the elimination of microbiological release testing when appropriate manufacturing process and raw materials control has been demonstrated. In-process steps that pose a challenge to microorganisms, such as heating, solvent application, and drying, that are routinely monitored can assure a safe and effective product and eliminate or reduce end-product testing. Appropriately monitored raw materials supplied from trustworthy suppliers help assure a consistent and predictable process. The FDA has approved reduced release testing for tablets and capsules in the review of new drug applications (NDAs) when sufficient process control has been demonstrated with consistent control of raw materials and the regulatory application contains sufficient data to support such a change.

Sterile Pharmaceutical Dosage Forms

Sterile dosage forms, such as powders for suspension and lyophilized products, have in general low water activity values. However, constitution or dilution of a powdered product with an aqueous solution creates a water activity which is conducive to microbial growth. For these products, the greatest risk of product contamination exists due to manipulations prior to patient administration. In some instances, numerous such transfers and manipulations may be required to obtain the final product concentration. The resulting aqueous suspension or solution may be stored at refrigerated or room temperature conditions prior to administration or between administrations to the patient.

Similar concerns exist for pharmacy bulk packages, sterile products that contain several single doses. These bulk packages are penetrated in a hospital pharmacy prior to distribution of the single doses into sterile containers. Sterility cannot be assured following penetration of the container-closure system. Even though aseptic technique is used, contamination is always possible due to human error. Therefore, there are limitations to the amount of time that the bulk package may be held following penetration. These time limits are approved by the FDA and stated in the product label. Microbial growth studies can provide regulators with necessary information in support of post-penetration storage periods and conditions.

Microbial Growth Potential and QbD—Examples and Case Studies

Understanding the microbial growth potential of a product solution can be beneficial for sterile products as well as nonsterile products and can affect the development of the product formulation and the design of the manufacturing process. The following examples and case studies demonstrate the usefulness of understanding microbial growth potential for process design and control and product use as described in the product labeling.

Examples and Case Studies

1. Moist heat sterilization—type of cycle and validation approach: Instead of employing moist heat sterilization cycles with high heat input, low heat input, bioburden-based validation approaches can be followed for a product that does not support microbial growth. Currently, two different ap-

proaches exist for sterilizing products on the market. There are multiple products sterilized using an "overkill" approach or cycles delivering excess lethality and validated using biological indicators with many orders of magnitude higher resistance than common bioburden. Alternatively, there are commercial drug products rendered sterile by moist heat terminal sterilization using bioburden-based or bioburden-biological indicator combination approaches delivering low but adequate lethality to assure sterility and safety.

Terminal sterilization assures a lower probability for a nonsterile unit in a defined load under defined parameters and is less costly than aseptic processing. However, there are commercial products that are aseptically filled even though they can be terminally sterilized without any adverse effects on their stability profile. One reason is that only the high heat input approach was evaluated during development without consideration of bioburden or bioburden-biological indicator validation approaches. By understanding the microbial growth potential of a product formulation, the most heat-resistant bioburden can be selected and used in sterilization cycle development or be compared to the biological indicator. There are approved sterilization cycles that have been developed using biological indicators other than *G. stearothermophilus.*

Exploring all options for moist heat sterilization during development is QbD because it leads to a scientific and risk-based sterilization method and cycle type that is validated using the most reasonable and risk-based approach. The most appropriate sterilization cycle is developed and validated for commercial manufacture.

It is worth noting that there are several commercial drug products including large volume parenterals that are sterilized using moist heat and low heat input cycles—for example, dextrose and salt solutions sterilized with minimum lethality (F_0) of less than 10 min—that have been approved for parametric release. For these products, sterility testing of each lot has been replaced with meeting defined sterilization process parameters (11). If it is possible to sterilize a growth-promoting solution such as dextrose using very low heat input and parametric release, then there are many possibilities for solutions that are not growth-promoting.

2. Increased batch size and holding times for aseptic processing or multiple campaigns: A longer bulk solution holding time can be applied if the product solution does not support microbial growth. However, good manufacturing practices still must be followed along with appropriate process control. This information should not be used to justify and hide inappropriate practices.

   In this example, the holding time of a sterile-filtered bulk solution between the end of filtration and start of filling was increased by more than 300% and up to five filling campaigns (consecutive batches) were approved. This was an interesting case study and may not be common due to extenuating circumstances of the formulation and manufacturing process: the product was highly bactericidal and toxic with a low pH and water content and was manufactured using distillation and redundant sterile filtration. Filling took place inside an isolator. The holding time and multiple campaign changes were validated by media simulations. The case study reveals that process improvements can be achieved if the microbial growth potential of the formulation is known and the aseptic manufacturing process is adequately controlled and validated. Had the firm understood the microbial growth potential of the formulation, these changes may have been incorporated into the manufacturing process at the time of submission of the original application.

3. Preservatives: Preservatives are added to multiple-dose drug product formulations for nonsterile products and during use of sterile products for the purpose of maintaining their microbiological quality over their shelf life (USP ⟨51⟩) (8). However, it is known that exposure to a preservative may lead to adverse events and allergic reactions in users (12–14). Consequently, for patient safety, it is desirable to use as low a concentration of the given preservative in the formulation of a drug product as possible. This is presented from a microbiological point of view and not a chemistry or stability point of view, as it is well known that preservative amounts may decrease significantly over time depending on storage conditions.

   An understanding of the drug product's microbial growth potential can aid the drug developer in determining both the selection of the preservative system and the safest preservative concentration to

incorporate into the drug formulation, while still allowing the drug product to meet the preservative effectiveness acceptance criteria in USP ⟨51⟩ (8). This approach to preservative system design is different than simply selecting the preservative concentration that was used in the last approved product for the same indication as the drug under development, a seemingly common practice in NDA product applications. Alternatively, the use of a preservative system has also been noted in single-dose products approved in the past. Current thinking and knowledge would not allow a preservative in a single-dose formulation without a valid rationale. Furthermore, a preservative should never be used to justify inappropriate, unhygienic, or non-validated manufacturing practices. For example, a single-dose sterile liquid product should not be preserved to justify a poorly controlled aseptic manufacturing process where there are media fill failures and possibly contaminated product. In this case, the process and practices should be revisited and modified instead of adding a preservative to the formulation.

It is worth noting that propofol, a drug used for general anesthesia, is a drug product with very high microbial growth potential. Adverse events were initially attributed to improper practices such as preparing syringes in batches for use throughout the day, reusing solutions or infusion pump lines on different patients, failure to wear gloves or disinfect the vial stoppers of the 50 mL and 100 mL vials prior to use (15, 16). The propofol formulation did not contain a preservative. Later, it was found that even single-dose vials were mishandled and used in multiple patients. Following these adverse events, sodium metabisulfite, disodium edetate, and other compounds have been added to the formulation to reduce the rate of microbial growth. However, even with these changes, the formulation does not meet the USP criteria for preservative effectiveness. Propofol is an emulsion that consists of soybean oil and/or egg yolk phospholipids that are highly conducive to microbial proliferation following introduction of microorganisms into the sterile product. For this drug, microbial growth studies performed during product development may have led to a different initial formulation, or to approval of the product with the current, strict handling instructions in the package insert and product label. The label has been revised multiple times, including as recently as this year (2010). While mishandling and inappropriate aseptic practices by hospital personnel contributed to the clinical adverse events, perhaps some of these events could have been prevented if the label included the current strict provisions upon approval of the drug, if a different formulation had been developed, or if vials containing low volumes of drug had been marketed so as not to allow for multiple use of a single vial. Subsequent microbial growth studies were conducted, and now the label instructs users that the formulation is inhibitive to microorganisms for up to 12 h in the event of external contamination.

4. In-use storage period prior to administration: Two case studies are presented here where microbial challenge studies resulted in additional product knowledge and optimization with labeling consequences.

    a) A lyophilized product that contained a significant amount of lactose was to be reconstituted and further diluted with sterile saline to a final concentration of 1 mg/mL and stored for 24 h at room temperature prior to administration. Reconstituted product was inoculated with very low counts of *Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus.* The comparison controls were tryptic soy broth (TSB) with product (1 mg/mL), TSB without product, saline without product, and negative controls (samples that were not inoculated). Two temperature conditions were evaluated: 2–8 °C and room temperature. The bacterial count was determined at initial time, 4, 6, 8, 12, 20, and 24 h. Product in saline was bactericidal to *P. aeruginosa* and bacteriostatic to *E. coli* and *S. aureus* at both temperature conditions. Product in TSB at 2–8 °C resulted in <0.5 $\log_{10}$ increase for all organisms. At room temperature, two of the challenge bacteria (*P. aeruginosa* and *E. coli*) increased >3 $\log_{10}$ after 24 h in the TSB product. TSB without product was bacteriostatic at 2–8 °C. Similar results were obtained with saline without product. As a result of these studies, the proposed in-use storage time of 24 h at room temperature was approved when reconstituting the product with saline.

    b) A lyophilized product was to be reconstituted with different diluents such as sterile water for injection (WFI), 0.9% sodium chloride injec-

tion, and Lactated Ringer's injection, stored at 2–8 °C for 5 days, and then further diluted and stored at room temperature for 48 h prior to administration. Data were provided to support the 5 day storage period at refrigerated conditions. Additional studies were performed to support storage at room temperature as requested by the NDA reviewer. One product vial was diluted with 100 mL of each diluent and inoculated with approximately 10–50 colony-forming units per milliliter (CFU/mL) of each microorganism (*Aspergillus niger, Candida albicans, E. coli, P. aeruginosa,* and *S. aureus*). Aliquots were withdrawn and tested at initial time, 24, 36, 48, 60, 72, 84, and 96 h. Similar results were obtained for sterile WFI and saline. *A. niger* remained relatively stable over the course of the study. Bacterial counts steadily decreased to the point of no detection. *C. albicans* was not detected in sterile WFI after 72 h, while there were survivors in saline. Similar results were obtained for the yeast and mold in product diluted with Lactated Ringer's. However, the results were different for the bacterial organisms. *S. aureus* numbers decreased to 1 CFU/mL at 24 h and the organism was not detected again until 96 h, at which point very high growth was obtained. *E. coli* started growing before the 48 h interval and reached high numbers after 72 h. *P. aeruginosa* started growing after 60 h. The study was repeated with only *E. coli* and *P. aeruginosa* and high growth was obtained for both organisms after only 24 h. As a result of these studies, only sterile WFI and 0.9% sodium chloride injection were selected for drug product reconstitution and dilution. Lactated Ringer's was removed from the label as a recommended diluent.

5. Extended pharmacy bulk holding times: Pharmacy bulk packages contain multiple single doses of a given sterile product. The purpose of the pharmacy bulk package is to dispense product into empty, sterile syringes in a clinical pharmacy. According to USP ⟨1⟩, the pharmacy bulk package closure may be penetrated only once. A sterile transfer device is then used for the distribution of each single use dose, and the pharmacy bulk package is exempt from the requirement that the product "contain a substance or suitable mixture of substances to prevent the growth of microorganisms." Because the product does not contain a preservative system, the post penetration holding period during which the product may be dispensed into sterile syringes is limited by FDA during the review of the product application.

   A product with a pharmacy bulk package-approved, post-penetration holding time of 4 h was subsequently approved for an extended holding time of 10 h after submission of data in a supplemental application demonstrating that the product does not support microbial growth. Challenge microorganisms (*S. aureus, P. aeruginosa, E. coli, C. albicans,* and *A. niger*) satisfied the USP ⟨51⟩ definition of no increase in growth when inoculated in the product and held at the intended storage conditions over the proposed extended holding period. The submission of this study led to approval of the extended post-penetration holding period of 10 h.

6. Therapeutic drug proteins and process design: This section presents a case study from an actual application of employing the knowledge gained from microbial growth studies to design the manufacturing process. Biological products are generally growth-promoting because they are derived from living systems. This is especially true of proteins in buffer matrices. However, understanding the level of growth promotion can result in the design of a more robust process. In this case, microbial studies were performed using the bulk solution and different concentrations of protein in the buffer matrix. During routine manufacture, the formulation is compounded and filtered after thawing and pooling of the drug substance. The holding time of the resulting bulk solution is a critical parameter of the aseptic process.

   A rather high inoculum was used for the microbial challenge studies in the range of 10^4–10^5 CFU/mL. Different drug substance concentrations were tested. The microbial count was evaluated at initial time, 6 h, 1, 3, 7, and 14 days for *P. aeruginosa, S. aureus, C. albicans,* and *A. niger. A. niger* counts remained static but there was formation of mycelia after 7 days. *C. albicans* had increased in numbers by Day 1 and continued to increase >10-fold after 3 days. *S. aureus* numbers remained static up to 3 days and then started decreasing. *P. aeruginosa* increased >10-fold after 1 day of incubation. As a result of

these studies, the maximum holding time from start of compounding until end of sterile filtration was limited to ≤12 h. These data demonstrated that the solution is highly growth-promoting and led to the design of a process with a pre-filtration step followed by sterile filtration. Additional holding time limits were built into the process for drug substance thawing and start of compounding to end of pre-filtration. It is worth noting that the applicant conducted the studies during development, effectively applying the QbD concept.

Summary

If microbial growth studies are performed during drug product development, the results can be submitted in the original application to allow for the proposed holding times. However, we rarely see these studies performed and submitted in the original application. As a result, these changes are commonly filed as post-approval Chemistry, Manufacturing, and Controls (CMC) supplements.

For nonsterile products that do not support microbial growth, justification can be provided in the regulatory application using the ICH Q6A (10) decision tree for reduced testing. It is recommended that historical data or appropriate studies are submitted along with a risk assessment analysis of the manufacturing process and raw materials.

The stringent manufacturing controls necessitated by aseptic processing have resulted in a proliferation of CMC supplements for manufacturing changes that may have been unnecessary had terminal sterilization and QbD principles been applied during drug product and process development. By developing a bioburden-based or bioburden-biological indicator sterilization cycle, a product that would normally be aseptically filled under the traditional paradigm could potentially be terminally sterilized, provided that the sterilization cycle does not cause stability or degradation issues. Lower heat input cycle development approaches may prevent these latter disadvantages associated with terminal processes and lead to optimization of sterilization processes.

Disclaimer

This article reflects the current thinking and experience of the authors. However, it is not an FDA policy document nor should it be used in lieu of regulations, published FDA guidance or direct communications with the Agency. No official support or endorsement by the FDA is intended or should be inferred.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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